Gel-Filtration applied to the Study of Lipases and Other Esterases

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The individual enzymes in mixtures of lipases and esterases are difficult to recognize, without prior separation, because of their low, overlapping substrate specificities. In anticipation of there being differences between the molecular weights of such enzymes, we have attempted their separation by gel filtration, and interpreted their gel filtration behaviour in terms of molecular weights (Andrews, 1964), as aids to their characterization. As tributyrin is hydrolysed by many lipases and esterases, tributyrin emulsion was used as substrate in most enzyme assays. Acid liberation was estimated by continuous titration.

Preparations of cows' milk tributyrinases, obtained by making milk 0·75M with NaCl and removing casein by centrifugation, were fractionated on a Sephadex G-200 column equilibrated with 0·75M-NaCl containing 0·025M-$MgCl_2$. Of three tributyrinases which together contributed a major part of the activity, only one (mol.wt. 112000) was clearly present in all the preparations examined, whereas the others (mol.wts. 75000, 62000) so varied in relative amounts that in some cases either one of them was barely detectable. Variations in the elution volume of a fourth tributyrinase, which also was found in each preparation, suggested that it was weakly adsorbed to the column, but even allowing for this it still seems to have a much lower molecular weight than do the other enzymes. Thus it may correspond to the lipase, mol.wt. 7000, recently isolated from milk clarifier slime by Chandan & Shahani (1963*a*, *b*).

Extracts of adipose tissue from individual female rats, when fractionated on a Sephadex G-100 column equilibrated with 0·2M-NaCl, gave tributyrinase elution diagrams which were more complex, and differed more widely one from another, than did those for the milk preparations. Six enzymes (mol.wt. about 200000, 94000, 68000, 55000, 47000, 39000) were detected overall, but whereas in some extracts the higher molecular weight ones predominated, in others the lower molecular weight ones were in excess.

In contrast, fractionation of wheat germ extracts, and assay with triacetin substrate, indicated the presence therein of only one esterase (cf. Mounter & Mounter, 1962) of mol.wt. 51000.

Although molecular weights estimated by the gel-filtration method are necessarily tentative, it does appear from our results that co-ordination of gel-filtration experiments with substrate and inhibitor studies will assist in characterizing the enzymes in mixtures of lipases and other esterases.

We thank Dr H. Gutfreund for his interest and advice. The investigations were supported by grant *FG-UK*-124-61, U.S. Department of Agriculture Public Law 480.

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